ORIGINAL PAPER

COMPLEX SPECTRAL CHARACTERIZATION OF ACTIVE PRINCIPLES FROM MARIGOLD (*CALENDULA OFFICINALIS*)

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Abstract*. The marigold is a very useful species of medicinal plants with many uses in phyto-therapy and cosmetics. The carotenoid pigments in the marigold's inflorescence represent a fundamental constituent of drugs. The pigment content was demonstrated and measured by thermal analysis, UV-VIS and FT-IR spectrometry. The results confirm the fact that this plant content important pigments useful for our healthy.*

Keywords*: Calendula oficinallis, carotenoids, chlorophyll, healthy, medicinal plants.*

1. INTRODUCTION

Calendula officinalis is an ornamental plant, used for landscaping, as a source of color in the gardens and as cut flowers and her dried petals are used as an herb tea medicinal or for cosmetically using [1]. This flower belongs to family *Asteraceae* and it is one of the valuable medicinal plants which contains oleanolic acid (or oleanic acid) and other compounds, which have considerable interest for potential health benefits, including protective effects against development of cancer, inhibition of existing tumor cells, protection against chemotherapy and radiation therapy adverse effects, anti-inflammatory activity, antioxidant activity, cardiovascular protective and antiviral effects. The petals colour is given by carotenoid pigments [2, 4].

The extract obtained from marigold posses a wide range of pharmacological effects. In internal use, marigold is useful in order to improve digestion and stimulate the production bile, healing gastric ulcers, etc. It has an anti-inflammatory as well as spasmolytic effect and is used with good effect for inflammation and small ulcer in the mouth and throat. In external use, marigold is beneficial in cases of burns, eczema, hemorrhoids and dry skin problems. The medicinal plants contain many active ingredients such as terpenoids, saponins, essential oil, organic acids and resins that act as antioxidants in the human body. The majority of the antioxidant capacity of medicinal plants may come from different compounds such as pigments: antocyanins, chlorophyll, carotenoids, flavonoids, flavones and vitamins C and E [3, 5].

The flavones are enhancing the ascorbic acid action by weakening the action of histamine through inactivation and protect the body against the toxic action of UV and X rays. In therapy, flavones are used under different medicine forms: ointments, tablets, or injection solutions. Making up the yellow pigments in flowers, leaves, and wood, flavones are widely used in analytic chemistry as colour and kellation reactants and in industry as coloured materials. In the food industry, they can be used as fat antioxidants [6].

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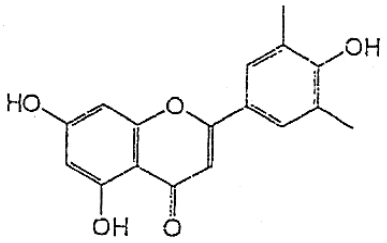
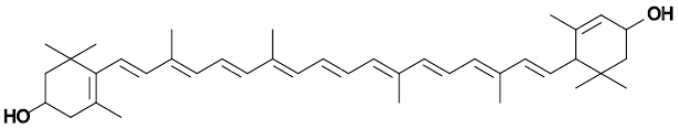
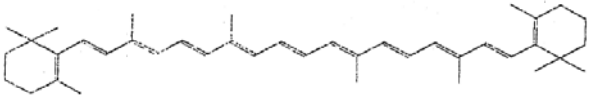


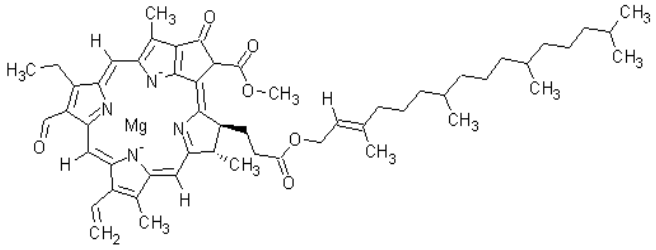
a) Flavones structure



b) Lutein structure



c) β-carotene structure



d) Chlorophyll B structure

Fig. 1. The chemical structure of carotenoids and chlorophyll measured and discussed in the paper: a) flavones; b) lutein; c) β-carotene; d) chlorophyll B.

2. MATERIALS AND METHOD

2.1 PREPARATION OF EXTRACTS FROM CALENDULA OFFICINALIS SAMPLES

The marigold (Fig. 2) petals and leaf were clean with distillated water, and then were dried 3 hours at 100°C in oven. The product (approx. 15 g) was extracted with 30 mL ethanol 95% by using Soxhlet method. The extract was filtered through a filter bed of cotton wool in a 100 mL volumetric flask and the filtrate was reintroduced in the recipient which contained the plant residue and then was extracted again, for 10 minutes, with 30 mL with initial solvent; the process has been repeated for several times. Finally, the extract was concentrated by using a rotary evaporator; the final extract reached a 10 mL volume. This filtrate was cooled at -4°C, and was kept in the dark.

Fig. 2. Marigold flower.

2.2. EXPERIMENTAL EQUIPMENTS

A M400 Carl Zeiss Jena UV spectrometer with: a 1 nm slit width, 1 nm step size, 0.3 nm/s average scan rate, deuterium lamp, double beam, microprocessor and quartz cell was used to measure the aqueous solution absorbance and the molar absorption spectra for the sample (at 22°C).

The sample was examined by using a Mettler 4000 TA, TG 50 thermal analyzer system at a rate of 10°C min^{-1} in a static air atmosphere; a Perkin-Elmer thermoanalyzer TG S-2 and DTA 500 at a rate of 20°C min^{-1} (Fig. 3). Also, a TG (Du PontTGA) thermobalance was connected to a PC running Du Pont data processing software. The used crucibles were from Al_2O_3 and the diameter was from 70 µL. About 20 mg of sample were subjected to analysis in a temperature range 25-500°C (20°C min^{-1}). Heating speed was 10 degrees 20°C/min. Liquid nitrogen's flow rate was set around 3 L/h.

Fig. 3. TGA thermal analyzer.

Fourier transformed IR spectroscopy (FT-IR) standard spectra were collected using a Perkin Elmer Spectrum GX spectrometer. Scans in the range of 400–4000 were accumulated for each spectrum at a spectral resolution of 4 cm^{-1}. It was possible to use the drift accessory with the powdered pure substance, thereby allowing for a better and easier analysis.

3. RESULTS AND DISCUSSION

All varieties of *Calendula* are rich in carotenoids. In our type of marigold, it was observed in the first stage, using the spectrophotometer method, spectra for lutein, a carotenoid pigment (446-450 nm) and chlorophyll b (648-655 nm), (Fig. 4).

Lutein is a xanthophyll and one of 600 known naturally-occurring carotenoid. This xanthophyll, like its sister compound zeaxanthin, has primarily been used as a natural colorant due to its orange-red color. As we know, lutein absorbs blue light and therefore appears yellow at low concentrations and orange-red at high concentrations [7].

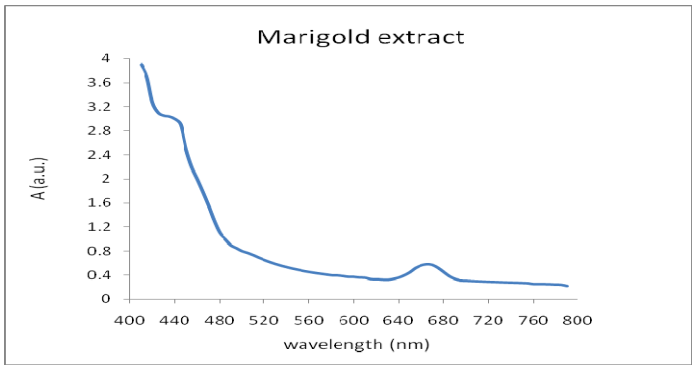


Fig. 4. UV-VIS spectra of marigold extract.

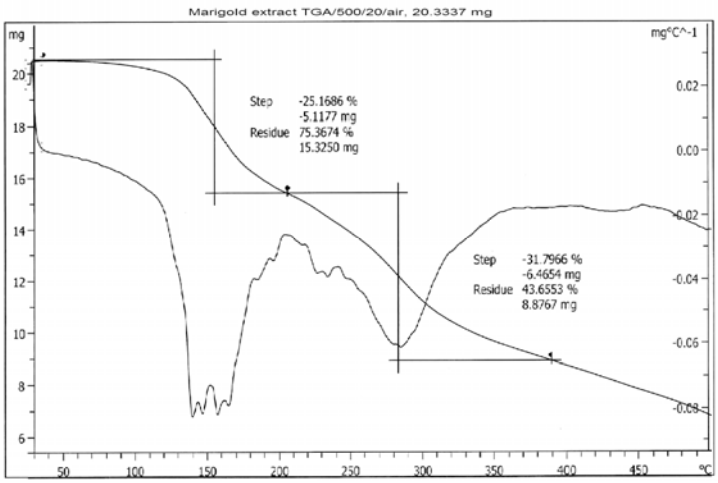


Fig. 5. TGA thermogram of marigold extract.

It was used liquid extract (obtained using Soxhlet method and then rotary evaporator to concentrate the solution). In thermogram (Fig. 5) it was observed very clearly the melting point of chlorophyll which was between 130°C and 140°C; for carotenoids, more accurate, for lycopene the melting point was observed between 172-175°C and for lutein, between 177°C - 178°C.

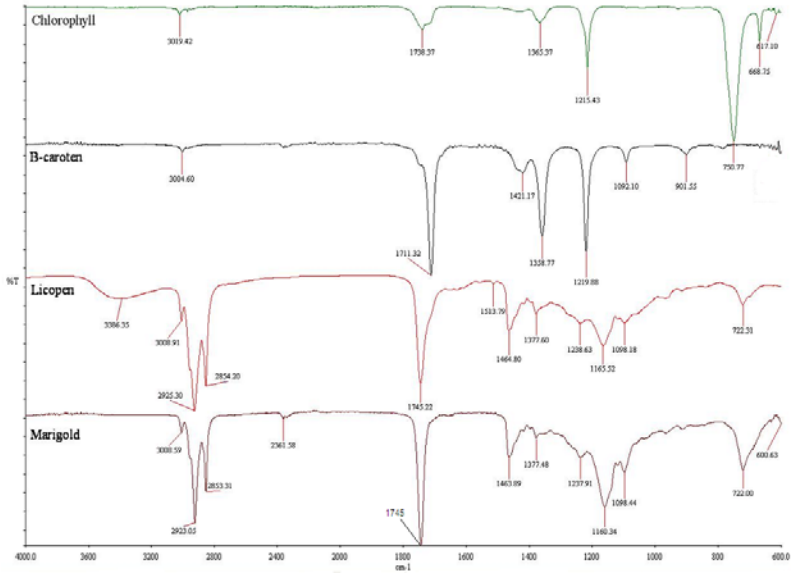


Fig. 6. FT-IR spectra using ATR method. Comparison between marigold extract, lycopene, beta-carotene and chlorophyll pigments.

Fig. 6 represents the comparison between marigold extract and lycopene, chlorophyll and beta-carotene pigments. Several intense bands (2923 cm^{-1}, 1745 cm^{-1}, 1464 cm^{-1}, 1160 cm^{-1}) in marigold have been observed and discussed using literature data.

In marigold extract it was observed a strong band at 1745 cm^{-1} and this band corresponding for lycopene and β carotene pigments, which means that these pigments prevail in *Calendula officinalis*.

It were observed a strong bands at 1463.89 cm^{-1} (antisymmetric band of methyl functional groups) which correspond for lycopene pigments (1464.80 cm^{-1}), at 1377.48 cm^{-1} (germinal methyl), 1238 and 1160.34 cm^{-1} (ether linkages), 1098.44 cm^{-1} (suggest the presence of flavones or terpenoids). The band at 1160 cm^{-1} can be attributed to a C–C stretching vibration of the carotenoid skeleton [8, 9].

The assignment of the band registered at 1464 cm^{-1} is confirmed by measurement on a lycopene standard obtained from tomato. Furthermore, measured spectra were shown a higher intensity band near 1464 cm^{-1} with a shoulder at 1377.48 cm^{-1} and that corresponds to higher amounts of β-carotene (1421.17 cm^{-1} with a shoulder at 1358.7 cm^{-1}) in comparison to lycopene (1464.8 cm^{-1} with a shoulder at 1377.6 cm^{-1}), which is reflected in the colour of this flower, as well. The principal firs peak 2923 cm^{-1} is attributed to -OH bands.

4. CONCLUSIONS

Marigold is one of the most valuable medicinal plants because a variety of phytochemicals such as, terpenoids, flavonoids, coumarins, quinones, volatile oil, carotenoids and other compounds are present in this plant.

The extracts of Calendula officinalis have a lot of pharmacological benefits. It exhibits several pharmacological activities such anti-HIV, anti-cancer, anti-inflammatory, hepatoprotective, spasmolytic and spasmogenic etc.

So, it is an important medicinal plant for mankind. Cultivating this species allows agriculturists to get quality yields with high content of active principles that result in considerable incomes. As many others medicinal plants, *Calendula officinalis L*. can bring an important profit to Romanian agriculture if is well cultivated. But, actually, we don't know yet, or we don't give importance to these natural treasures that we have.

The literature confirm the fact that lutein was found in marigold flower petals [10].

The pigments extracted from marigold leaves, showed peaks at specific wavelengths of chlorophyll (648-655 nm) and a carotenoid pigment, lutein (446-450 nm).

In this paper, it was demonstrated presences of different types of pigments using thermal analysis, UV-VIS, FT-IR spectrometry. Future experiments will demonstrate the benefits of these pigments in medicine, for human health.

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